METHOD FOR THE SYNTHESIS OF DNA FRAGMENTS

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Abstract of **WO0075368**

The invention relates to a method that can be carried out in parallel and automated for the production of any nucleic acid, comprising the following steps: a) coupling an oligonucleotide to a solid matrix; b) adding an additional oligonucleotide; c) performing ligation of the oligonucleotide from steps a) and b) in an orientation; d) removing excess reactants and enzymes from the reaction preparation; e) effecting cleavage of the ligation product from step c) with a restriction system that cleaves outside the recognition sequence, whereby cleavage is effected in the shortened or lengthened oligonucleotide from step a) or in the oligonucleotide from step b); f) separating the reaction mixture from the lengthened or shortened oligonucleotide from step a); g) repeating at least one steps b) to f); h) performing successive sequence-independent linkage of the fragments obtained after executing steps a) to g) until the desired product is obtained.

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Method to the synthesis of DNA fragments after < RTI ID=1.1> derzeitigen< /RTI> State of the art < RTI ID=1.2> müssen< /RTI> < RTI ID=1.3> für< /RTI> a synthesis of one about 2.5 KB large nucleic acid sequence first about 50 different, partly overlapping approx. < RTI ID=1.4> 80mer< /RTI> Oligonukleotide to be synthesized and up-cleaned. These are hybridized then in pairs or in Subsets and < by means of a Klenow polymerase reaction; RTI ID=1.5> aufgefüllt< /RTI> or with the Oligonukleotiden as primer in a polymerase nuclear chain reaction (PCR), lying outside, manufactured and (usually < RTI ID=1.6> über< /RTI> restriction places which can be inserted) < unidirectionally with one another; RTI ID=1.7> verknüpft.< /RTI> This method is as " Gap filling " method well-known. Gene fragments can alternative be synthesized by enzymatic or chemical Ligation; these fragments can be built up then after Aufreinigung and/or Klonierung to larger gene sections (so-called.

< RTI ID=1.8> Cartridge method). < /RTI> Both procedures < RTI ID=1.9> erfordern< /RTI> ideally at least one week, as a rule however rather 6-12 weeks, every now and then even 6 months. Sequenzielle, at solid phases bound methods only < because of the multiplicity of the necessary reaction steps; RTI ID=1.10> geringe< /RTI> Yields and are also very much < therefore; RTI ID=1.11> fehleranfällig.< /RTI>

One of the Hauptprobleme consists of the fact that < RTI ID=1.12> längere< /RTI> Oligonukleotide out < RTI ID=1.13> Gründen< /RTI> the coupling efficiency, which reaches 99% per step even with well running syntheses only, always one < RTI ID=1.14> unvermeidbaren< /RTI> Portion < on; RTI ID=1.15> Abbruchprodukten< /RTI> exhibit. Beyond that it comes also to Deletionen, which does not < out; RTI ID=1.16> 100% igem< /RTI> Capping result. Even with very good syntheses this portion is with approx. 0.25% per coupling step. Also the separation of the Tritylschutzgruppen after completion of the synthesis < RTI ID=1.17> verläuft< /RTI> does not < RTI ID=1.18> vollständig.< /RTI> The incomplete Oligonukleotidprodukte developed in such a way can even at large expenditure of < RTI ID=1.19> längeren< /RTI> Oligonukleotiden does not < RTI ID=1.20> vollständig< /RTI> are separated.

With an average coupling efficiency of 98% < RTI ID=1.21> erhält< /RTI> one for example with a 80mer a yield of the desired product with full length of only 19,86%. With the nowadays available Aufreinigungsverfahren the desired final product in one can Purity of at best 95% to be represented. Even if then only a small part of the final-cleaned Oligonukleotide is incorrect, rises nevertheless < RTI ID=2.1> Wahrscheinlichkeit< /RTI> an incorrect final sequence with the number of the assigned Oligonukleotide dramatically on. A sequence, which of 50 the described Oligonukleotide consists, is correct therefore only in 7,7% of all cases and must be done over again therefore in all rule. A relatively rare installation of wrong bases due to false couplings during the synthesis is not < thereby; RTI ID=2.2> berücksichtigt.< /RTI>

Due to that variety < RTI ID=2.3> möglicher< /RTI> Sequences themselves relatively short Oligonukleotide (already of a 30mer exist < RTI ID=2.4> über< /RTI> < RTI ID=2.5> 1018< /RTI> < RTI ID=2.6> mögliche< /RTI> Sequence variants) it is besides practically < RTI ID=2.7> not possibly, < /RTI> To use Oligonukleotide for different Genkonstrukte again. Therefore it is technically not feasiblly, < RTI ID=2.8> sämtliche< /RTI> to < RTI ID=2.9> Generierung< /RTI> arbitrary sequences < RTI ID=2.10> benötigte< /RTI> To reproach Oligonukleotide. For each new Genkonstrukt < RTI ID=2.11> müssen< /RTI> new in each case Oligonukleotide to be synthesized and up-cleaned. Only a fraction of the synthesized material is however < RTI ID=2.12> tatsächlich< /RTI> < RTI ID=2.13> für< /RTI> the gene synthesis assigned, the remainder can do out described above < RTI ID=2.14> Gründen< /RTI> not to be used. Those does not < RTI ID=2.15> gelöste< /RTI> Integration of the Oligonukleotidsynthese and their Aufreinigung into the gene synthesis process is < one; RTI ID=2.16> Main obstacles, < /RTI> why one < RTI ID=2.17> vollständige< /RTI> Automation of this process technically only extremely with difficulty and practically probably < at present; RTI ID=2.18> überhaupt< /RTI> to realize not.

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Those the present invention < RTI ID=2.19> zugrundeliegende< /RTI> Task exists thus in the supply of a method to the efficient synthesis < RTI ID=2.20> doppelsträngiger< /RTI> DNA fragments of arbitrary sequence and length. A further task consists of placing a method ready which it permits to set arbitrary DNA molecules from a limited library of basic modules together. A further task consists of pointing a method out which < the parallel synthesis and the sequence-independent; RTI ID=2.21> Verknüpfung< /RTI> arbitrary gene fragments permits. The fulfilment of these two conditions is necessarily < RTI ID=2.22> für< /RTI> < RTI ID=2.23> Realisierung< /RTI> a complete automation of the gene synthesis procedure. A further task exists in the supply of a kit to < RTI ID=2.24> automatisierten< /RTI> Preparation < RTI ID=2.25> doppelsträngiger< /RTI> DNA fragments.

The task is < RTI ID=2.26> gelöst< /RTI> by the supply of a method to the preparation of a nucleic acid molecule, the comprising steps: a) Coupling of a Oligonukleotids with an end to a solid matrix, whereby those
Coupling over a modification takes place, and the Oligonukleotid a recognition sequence < RTI ID=3.1> für< /RTI> a TypIIS restriction enzyme < RTI ID=3.2> contains, < /RTI> which outside of its
Recognition sequence cuts, b) addition of a further, at least partly < RTI ID=3.3> doppelsträngigen< /RTI> Oligonukleotids with another recognition sequence < RTI ID=3.4> für< /RTI> a TypIIS restriction enzyme, which cuts

outside of its recognition sequence, as in step A), whereby this
Oligonukleotid to the matrix to bind does not know, C) Ligation of the Oligonukleotide from step A) and b) in orientation not given by the blocking that to ligierenden ends, D) removing not spent reactant as well as enzymes, e) cracking of the Ligationsprodukts from step C) with a TypIIS restriction enzyme, which cuts outside of its recognition sequence, whereby the cracking in
Oligonukleotid from step A) takes place, f) separating of the received nucleic acid molecule of the reaction mixture.

Furthermore the task is < RTI ID=3.5> gelöst< /RTI> by the supply of a method to the preparation of a nucleic acid molecule, the comprising steps: a) until D) like above, e) cracking of the Ligationsprodukts from step C) with a TypIIS restriction enzyme, which cuts outside of its recognition sequence, whereby the cracking in that
Nucleic acid sequence of the Oligonukleotids from step b) takes place,
Separating of the reaction mixture of the elongated Oligo nucleotide from step A, received in step e)), g) at least unique repeating of the steps b) to < RTI ID=3.6> f). < /RTI>

Furthermore the task is < RTI ID=3.7> gelöst< /RTI> by the supply of a method to the preparation one < RTI ID=3.8> Nucleic acid molecule, < /RTI> the comprising steps: a) until g) like above, h) cracking of the received nucleic acid molecule with a TypIIS restriction enzyme, which cuts outside of its recognition sequence, whereby the cracking in Oligonukleotid from step A) takes place, and if necessary i) cracking of the received nucleic acid molecule with a TypIIS restriction enzyme, which cuts outside of its recognition sequence, whereby the cracking in
Oligonukleotid from step b) takes place.

Preferentially a method, whereby after step C) is < as step; RTI ID=4.1> C) 'one Exonuklease and/oder< /RTI> Phosphatase reaction is accomplished. Furthermore a method is preferential, whereby the reaction mixture of the step < RTI ID=4.2> C) 'nach< /RTI> the reaction < RTI ID=4.3> entfernt< /RTI> wird. Furthermore a method, whereby in the last repetition of the steps b) to f) the step e is preferential) is not accomplished. Furthermore a method, whereby received nucleic acid by restriction splitting of the Oligonukleotid from step A) is preferential is separated. Furthermore preferentially a method, whereby the coupling of the Oligonukleotids from step A) is < to the solid matrix; RTI ID=4.4> über< /RTI> a modification takes place. A method is particularly preferential, whereby the modification < RTI ID=4.5> Biotinrest, < /RTI> a Digoxigeninrest, a Fluoresceinisothiocyanat (FITC), revision modification November heiress dung or a Succinylester is. Furthermore a method is preferential, whereby the Oligonukleotid from step A) and/or b) < RTI ID=4.6> über< /RTI> a loop < RTI ID=4.7> verfügt.< /RTI> A method, whereby the Oligonukleotid from step A) is particularly preferential with the loop is coupled to the solid matrix. A method is particularly preferential, whereby the solid matrix < RTI ID=4.8> Kügelchen< /RTI> (bead), preferably from glass or polystyrene, < RTI ID=4.9> Microscope slide, < /RTI> a DNA chip, the indentation of a micro titer plate (wave) or a reaction tube is. Insbesondere bevorzugt ist ein Verfahren, wobei die feste Matrix einen Streptavidinrest, einen <RTI ID=4.10>anti-Digoxigenin-Antikörper</RTI> or an anti- FITC antibody covers. Furthermore a method is preferential, whereby the Oligonukleotide from step A) and b) at their to ligierenden ends < RTI ID=4.11> über< /RTI> complementary < to each other; RTI ID=4.12> Einzelstrangüberhänge< /RTI> order.

A method is particularly preferential, whereby the single strand overhangs < RTI ID=4.13> 1,2,3,4< /RTI> or 5 nucleotides are long. A method is particularly preferential, whereby < RTI ID=4.14> synthetisierte< /RTI> Nucleic acid in a locking step with one < RTI ID=4.15> replikationsfähigen< /RTI> DNA (a Plasmidvektor, one phage or < RTI ID=4.16> Virus DNA, < /RTI> a artifiziellen Chromosom, a PCR product or a further synthetic manufactured DNA < RTI ID=4.17> verknüpft< /RTI> becomes. Particularly preferentially a method is < to the making of Codon optimized open reader aster, to the purposeful Mutagenese of promoters; RTI ID=4.18> Enhancern< /RTI> or DNAs, which code for proteins. In particular preferentially the use is < RTI ID=4.19> erfindungsgemässen< /RTI> Nucleic acid as Codon optimized DNA Vakzine, to the mutation analysis of protein domains, when stencil for designer proteins, when Expressionskonstrukt < RTI ID=4.20> for in vitro< /RTI> Protein synthesis, to the preparation of Ribozymen or Aptameren, as sonde to the proof of pathogener microorganisms, as sonde to the detection of the expression of genes, to the proof of allele-specific mutations, to the detection of protein/protein connection, protein/Peptid-Bindung < RTI ID=5.1> und/oder< /RTI> the connection of low-molecular materials to proteins.

Furthermore the task is < RTI ID=5.2> gelöst< /RTI> by the supply of a kit to the preparation after a nucleic acid < RTI ID=5.3> erfindungsgemässen< /RTI> Method, comprising A) a library of < RTI ID=5.4> 1< /RTI> until 1.048.576 different Oligonukleotiden < RTI ID=5.5> contains, < /RTI> whereby the Oligonukleotide < RTI ID=5.6> über< /RTI> a modification at an end to a solid matrix can be linked and the Oligonukleotid a recognition sequence or a part that Recognition sequence < RTI ID=5.7> für< /RTI> < RTI ID=5.8> TypIIS restriction enzyme contains, < /RTI> which cuts outside of its recognition sequence, b) a further library from 4 to 1.048.576 different Oligonukleotiden < RTI ID=5.9> contains, < /RTI> whereby everyone the Oligonukleotide a recognition sequence < RTI ID=5.10> für< /RTI> a TypIIS
Restriktionsenzym, welches ausserhalb seiner Erkennungssequenz schneidet, <RTI ID=5.11>enthält,</RTI> the fact that of the TypIIS restriction enzyme from A) is different, and if necessary the other part of the recognition sequence of the restriction enzyme from step A) < RTI ID=5.12> contains, < /RTI> c) a solid matrix, D) of reservoirs < RTI ID=5.13> für< /RTI> the preparation of the nucleic acid molecule < RTI ID=5.14> benötigten< /RTI> Enzymes and/or other reagents.

A kit is preferential, whereby the enzymes a ligase or a Topoisomerase < RTI ID=5.15> und/oder< /RTI> or several restriction enzyme (E) and/or a Exonuklease and/or a Phosphatase cover.

An apparatus, which < after input of the desired cousin sequence, is particularly preferential; RTI ID=5.16> sämtliche< /RTI> Reaction steps solid put and < RTI ID=5.17> selbsttätig< /RTI> to process can.

The invention is < by the following figures; RTI ID=5.18> erläutert.< /RTI>

Figure < RTI ID=5.19> 1< /RTI> a schematic display shows < RTI ID=5.20> erfindungsgemässen< /RTI> Method. Bio means a modification (z. B. Biotin), with that the Anchor Oligonukleotid to a solid matrix (z. B.

Streptavidin) is coupled. T, G, C, A and N designate the Nukleinsäurebasen, whereby T Thymus DIN, G guanine, C

cytosine, A adenine and N one arbitrary of the four Nukleinsäurebasen means.

Figure 2 shows schematically the structure one < RTI ID=6.1> EasyPro< /RTI> Transkriptions/lateral adjustment system of PCR fragments. Bio means a modification, with which the Anchor Oligonukleotid is coupled to a solid matrix. < RTI ID=6.2> 5-UTR< /RTI> meant < RTI ID=6.3> 5 ' - untranslatierter< /RTI> < RTI ID=6.4> Bereich.< /RTI> ATG means Startcodon. a sixfold lining up of Histidincodons means 6 x His. Single T overhang means one < RTI ID=6.5> Überhang< /RTI> of a thymus DIN remainder.

Figure 3 shows a schematic display of a mini reactor < RTI ID=6.6> für< /RTI> the protein synthesis.

Figure 4 shows a schematic display of the production of a Peptidlibrary with < RTI ID=6.7> QuickPepTM Verfahren.< /RTI> Bio means a modification, with which the Anchor Oligonukleotid is coupled to a solid matrix. T7 means T7-Promotor. rbs internal Ribosomenbindungsstelle means. ATG means Startcodon. EK means Enterokinase interface.

Peptide ORF means open of the peptide. STOP means the Stopcodon. Poly A designates the poly A tail.

Figure 5 shows a schematic display of the selection of Ribozymen with < RTI ID=6.8> Ribo S electTM Verfahren.< /RTI>

Figure 6 shows a schematic display of the proof from Pathogenen to enrichment by PCR < RTI ID=6.9> (PathoCheckTM). < /RTI> Bio means a modification, with which the Anchor Oligonukleotid is coupled to a solid matrix.

Figure 7 shows a schematic display of the identifying of well-known alleles by Ligation of labeled Splinker < RTI ID=6.10> (LIMAm). < /RTI> Bio means a modification, with which the Anchor Oligonukleotid is coupled to a solid matrix. x means the place, in which the modification which can be determined is present.

Figure 8 shows a schematic display of the parallel analysis of < RTI ID=6.11> MRNA Arrays< /RTI> (PAMINATM).

Figure 9 shows the schematic display of a Anchor Oligonukleotids. Bio means a modification, with which the Anchor Oligonukleotid is coupled to a solid matrix. T, G, C, A designate the Nukleinsäurebasen, whereby T thymus DIN, G guanine, C cytosine, A adenine means. < RTI ID=7.1> Esp3I< /RTI> marks a restriction enzyme.

Figure 10 shows the schematic display of a Anchor Oligonukleotids. Bio means a modification, with which the Anchor Oligonukleotid is coupled to a solid matrix. T, G, C, A designate the Nukleinsäurebasen, whereby T thymus DIN, G guanine, C cytosine, A adenine means. BpiI designates a restriction enzyme.

Figure 11 shows the schematic display of a Bipartite Anchor Oligonukleotids. Bio means a modification, with which the Anchor Oligonukleotid is coupled to a solid matrix. T, G, C, A designate the Nukleinsäurebasen, whereby T thymus DIN, G guanine, C cytosine, A adenine means.

Figure 12 shows the schematic display of a Splinker Oligonukleotids. T, G, C, A designate the Nukleinsäurebasen, whereby T thymus DIN, G guanine, C cytosine, A adenine means. BsaI and < RTI ID=7.2> Eco311< /RTI> mark restriction enzymes.

Figure 13 shows the schematic display of a Bipartite Splinker Oligonukleotids. T, G, C, A designate the Nukleinsäurebasen, whereby T thymus DIN, G guanine, C cytosine, A adenine means. BsaI and < RTI ID=7.3> Eco311< /RTI> mark restriction enzymes.

Figure 14 shows a schematic display of the synthesis way < RTI ID=7.4> längerer< /RTI> < RTI ID=7.5> Nukleinsäuren< /RTI> with < RTI ID=7.6> erfindungsgemässen< /RTI> Method. The bars < RTI ID=7.7> symbolisieren< /RTI> < RTI ID=7.8> doppelsträngige< /RTI> DNA fragments, which < parallel through one behind the other switched; RTI ID=7.9> Ligations/Restriktionszyklen< /RTI> were synthesized. In the final product neighbouring < RTI ID=7.10> Teilabschnitte< /RTI> by the Ligation of a aufligierten Splinkers with a aufligierten Anchor are connected in each case. In such a way won larger fragments are then < in; RTI ID=7.11> nächsten< /RTI> Step again either with the Anchor specific or the Splinker specific Restriktionsendonuklease cut and < RTI ID=7.12> über< /RTI> complementary overhangs < with one another; RTI ID=7.13> verknüpft< /RTI> etc., so that the length of the fragments with each step doubles itself. The linkage is perfectly sequence independent, since the recognition sequences of the used Restriktionsendonukleasen are built in the parts of the aufligierten fragments cut off in each case to lie and therefore not into increasing nucleic acid.

The numbers over the bars mean the value of the fragments in pairs of cousins. On the basis of 20 pair of cousins large DNA fragments results thus after four Transpositionen a max.

Length of 320 pairs of cousins, after five Transpositionen a length of 640 pairs of cousins, after six Transpositionen a length of 1280 pairs of cousins, after seven Transpositionen a length of 2560 pairs of cousins, etc.

" Parallel " or " parallel synthesis " it means definitions the term used here that different < RTI ID=8.1> erfindungsgemässe< /RTI> Nucleic acid molecules simultaneous in separate reaction beginnings to be synthesized can, around then with < RTI ID=8.2> erfindungsgemässen< /RTI> Method z. B. as Anchor or Splinker to elongated nucleic acid molecules to be ligiert < RTI ID=8.3> können.< /RTI>

The term " Sloning " (sequential Ligation of Oligonukleotiden in sequence-independent way), used here, refers to a method to the successive Ligation of Oligonukleotiden with arbitrary sequence.

Used the here < RTI ID=8.4> Term " Anchor " or " Anchor Oligonukleotid " bezieht< /RTI> itself on a Oligonukleotid, which < RTI ID=8.5> über< /RTI> a modification to a solid matrix to be coupled can. In the sense the Oligonukleotid contains of the present invention in its < RTI ID=8.6> doppelsträngigen< /RTI> Portion furthermore a restriction interface for a TypIIS restriction enzyme, which cuts outside of its recognition sequence.

Used the here < RTI ID=8.7> Term " Splinker " or " Splinker Oligonukleotid " bezieht< /RTI> itself on a Oligonukleotid, which < RTI ID=8.8> über< /RTI> none and/or. different one modification < RTI ID=8.9> ordered, < /RTI> so that it does not bind to the matrix, to which the Anchor Oligonukleotide is coupled.

The term " Dumbbell " used here (on dt. : < RTI ID=8.10> Glockenklöppel) < /RTI> refers to a DNA< RTI ID=8.11> Structure, < /RTI> by a doubling rank is characterized, which is flanked by two loops.

An aspect of the present invention concerns a method to the preparation of a nucleic acid molecule, the comprising steps: a) Coupling of a Oligonukleotids with an end to a solid matrix, whereby those
Coupling over a modification takes place, and the Oligonukleotid a recognition sequence < RTI ID=9.1> für< /RTI> a TypIIS restriction enzyme < RTI ID=9.2> contains, < /RTI> which outside of its
Recognition sequence cuts, b) addition of a further, at least partly < RTI ID=9.3> doppelsträngigen< /RTI> Oligonukleotids with another recognition sequence < RTI ID=9.4> für< /RTI> a TypIIS restriction enzyme, which cuts outside of its recognition sequence, as in step A), whereby this
Oligonukleotid to the matrix to bind does not know, D) Ligation of the Oligonukleotide from step A) and b) in orientation not given by the blocking that to ligierenden ends, h) removing not spent reactant as well as enzymes, i) cracking of the Ligationsprodukts from step C) with a TypIIS restriction enzyme, which cuts outside of its recognition sequence, whereby the cracking in
Oligonukleotid from step A) takes place, j) separating of the received nucleic acid molecule of the reaction mixture.

A further aspect of the invention relates to a method to the preparation of a Nukleinsäure< RTI ID=9.5> molecule, < /RTI> the comprising steps: a) until D) like above, e) cracking of the Ligationsprodukts from step C) with a TypIIS restriction enzyme, which cuts outside of its recognition sequence, whereby the cracking in that
Nucleic acid sequence of the Oligonukleotids from step b) takes place,
Separating of the reaction mixture of the elongated Oligo nucleotide from step A, received in step e)), k) < at least; RTI ID=9.6> einmaliges< /RTI> Repeat the steps b) to < RTI ID=9.7> f). < /RTI>

A further aspect of the invention relates to a method to the preparation of a Nukleinsäure< RTI ID=9.8> molecule, < /RTI> the comprising steps: a) until g) like above, h) cracking of the received nucleic acid molecule with a TypIIS restriction enzyme, which cuts outside of its recognition sequence, whereby the cracking in
Oligonukleotid from step A) takes place, and if necessary i) cracking of the received nucleic acid molecule with a TypIIS restriction enzyme, which cuts outside of its recognition sequence, whereby the cracking in
Oligonukleotid from step b) takes place.

One of the two in each reaction step to linking Oligonukleotide (the so-called.

< RTI ID=10.1> ?Anchor? - Oligonukleotid) < /RTI> is < RTI ID=10.2> über< /RTI> a modification, z. B. a low-molecular chemical connection such as biotin or Digoxigenin, to a solid matrix linked. In a preferential embodiment it concerns thereby magnetic Streptavidin coated or anti Digoxigenin coated Beads. The other Oligonukleotid (the so-called ?Splinker? Oligonukleotid) possesses also < RTI ID=10.3> blockiertes< /RTI> End, but no such or however different one modification. The point on it arrives is that that the Anchor Oligonukleotide is separated by the connection to a suitable matrix from the Splinker Oligonukleotiden < RTI ID=10.4> können.< /RTI> Therefore arbitrary connections z can do. B. Biotin, Digoxigenin, Fluoresceinisothiocyanat (FITC), revision modification November heiress fertilizing, Succinylester and other one the person skilled in the art < RTI ID=10.5> geläufige< /RTI> Connections, to be used, < RTI ID=10.6> sofern< /RTI> they suitably are direct, or indirect (z. B. over one < RTI ID=10.7> Antibody) < /RTI> to mediate a connection to a solid phase.

Anchor Oligonukleotide can consist either of a only one partially complementary Oligonukleotid, which < RTI ID=10.8> über< /RTI> a modification in the loop sequence to a solid phase can be linked preferably, or from two < RTI ID=10.9> einzelsträngigen< /RTI> Oligonukleotiden, which form a doubling rank, preferably with a single strand overhang. Since only one of the two < RTI ID=10.10> Stränge< /RTI> to a matrix to be coupled, can the other one must be denatured and separated if necessarily by alkali or heat (about over < as Template; RTI ID=10.11> für< /RTI> to serve a PCR reaction). In order to make sure that also with such split Anchor Oligonukleotiden only an end is ligierbar it is not <, for the Ligation; RTI ID=10.12> benötigten< /RTI> Ends accordingly blocks.

Nucleic acid sequences of exemplary Anchor Oligonukleotide are < Anchor A3I; RTI ID=10.13> 5 ' - GCTTCGAGACGCGTTTTCGCGTCTCG-3'& lt; /RTI> (SEQ ID NR: < RTI ID=10.14> 1< /RTI> ; FIG. 9) Anchor A2+ < RTI ID=11.1> 5 ' - AGAATGGTCTTCGAGCTTTTGCTCGAAGACCA-3'& lt; /RTI> (SEQ ID NR: 2 ; FIG. 10) Bipartite Anchor 5 ' - CGCGGATCCGCGGCGT-3' (SEQ ID NR: 3, FIG. 11) < RTI ID=11.2> 5 ' - CGAGACGCCGCGGATCCGCG-3'& lt; /RTI> (SEQ ID NR: 4, FIG. 11) Splinker Oligonukleotide can consist either of a only one partially complementary Oligonukleotid or of two < RTI ID=11.3> einzelsträngigen< /RTI> Oligonukleotiden, which form a doubling rank, preferably with a single strand overhang, D. h. one has an at least partial complementary pair of Oligonukleotiden, whereby those does not < in each case to ligierenden ends of the two; RTI ID=11.4> Einzelstränge< /RTI> blocked its < RTI ID=11.5> müssen.< /RTI> The preferential single strand overhang sequence must be more complementary to that to ligierenden Anchor Oligonukleotid in each case. Nucleic acid sequences of exemplary Splinker Oligonukleotide are < RTI ID=11.6> Splinker S 1H< /RTI> < RTI ID=11.7> 5-AAGCTTCTGGAGACCGCTTTTGCGGTCTCCAGAA-3< /RTI> (SEQ < RTI ID=11.8> ID< /RTI> NR: < RTI ID=11.9> 5, < /RTI> FIG. 12) Bipartite Splinker < RTI ID=11.10> 5-CTCGAAGCGGAGACCGCCAC-3< /RTI> (SEQ ID NR: 6, FIG. 13) 5 ' - GTGGCGGTCTCCGCTT-3' (SEQ < RTI ID=11.11> ID NR: 7, < /RTI> FIG. 13) Both Anchor like also Splinker Oligonukleotide can contain overhangs of a defined length, in a preferential embodiment one to < RTI ID=11.12> five Nukleotide.< /RTI> These overhangs are with that in each case to ligierenden Oligonukleotiden more complementary to each other, at 5 the ' end < RTI ID=11.13> phosphoryliert< /RTI> and can be ligiert only in an orientation with one another. A ligiertes Oligonukleotid with z develops. B. a so-called " Dumbbell " - structure. Around one < RTI ID=11.14> vollständige< /RTI> Ligation of all available Anchor Oligonukleotide to reach, can be added the anzuligierenden Splinker Oligonukleotide in 2 to 10fachem surplus. The surplus, non-reacted Splinker is away-washed after each Ligationsschritt with buffer. Become z. B. with Streptavidin coated magnetic Beads used, knows the Beads with < RTI ID=11.15> über< /RTI> a Streptavidin/Biotin connection bound Anchor Oligonukleotiden as well as the anligierten Splinkern by the inset of a magnet in the reaction beginning to be held back. Alternatively z can. B. directly with Streptavidin coated Wells, glass (beads), < RTI ID=12.1> Microscope slide, < /RTI> DNA chip or arbitrary other solid phases to be used. Beads are usually preferred, because them < a larger surface and therefore one; RTI ID=12.2> höhere< /RTI> < RTI ID=12.3> Bindungskapazität< /RTI>

exhibit.

In order to be able to accomplish further Ligationen, a recognition sequence must < RTI ID=12.4> für< /RTI> a Restriktionsendonuklease available its, which cuts the nucleic acid sequence outside of this recognition sequence in the anligierten Splinker Oligonukleotid. Examples < RTI ID=12.5> for solche< /RTI> Enzymes are < RTI ID=12.6> BpiI, < /RTI> < RTI ID=12.7> Esp3I, < /RTI> < RTI ID=12.8> Eco3lI, < /RTI> SapI etc. < RTI ID=12.9> Für< /RTI> < RTI ID=12.10> erfindungsgemässe< /RTI> Method < RTI ID=12.11> nützliche< /RTI> Restriction enzymes and their recognition sequences and interfaces are < in the Rebase data base under HTTP; RTI ID=12.12>: //rebase.< /RTI> neb. com/rebase/rebase. to find HTML. At the restriction interface contained in the Splinker Oligonukleotiden the Ligationsprodukte are cut in such a way that a part of the Splinkersequenz at the Anchor Oligonukleotid remains. Simultaneous one is produced by a sequence overhang which can be consulted for the Ligation of a further Splinker Oligonukleotids. The other abgespaltene part and not the ligierten remainder of the Splinker Oligonukleotids, the restriction enzyme as well as the restriction buffer are washed from the reaction beginning, on which a further cycle begins. The cycle can either only < RTI ID=12.13> einmal< /RTI> to be accomplished or however several times repeated, before < in such a way; RTI ID=12.14> verlängerten< /RTI> Oligonukleotide < RTI ID=12.15> ihrerseits< /RTI> with the parallel synthesized neighbour fragments < RTI ID=12.16> verknüpft< /RTI> become. Since with cutting edges with versc are favourable. In order to exclude non-converted Anchor Oligonukleotide from the further synthesis, fakultativ Phosphatase can be inserted a step after the Ligation Exonuklease and/or, whereby the overhang < or at least; RTI ID=13.1> für< /RTI> the following Ligation necessities < RTI ID=13.2> 5 ' - Phosphatgruppe< /RTI> < RTI ID=13.3> entfernt< /RTI> becomes. The portion of non-converted Anchor Oligonukleotiden is only small with an assigned surplus of Splinker Oligonukleotiden.

A far reaction should besides only then < RTI ID=13.4> möglich< /RTI> its, if the same sequence a further time is anligiert, why the danger of a contamination with is not to be regarded or only partly converted Anchor Oligonukleotiden than relatively small.

So the nucleic acid sequence aufligierte after several Ligations and restriction cycles knows afterwards by cutting edges with a restriction enzyme, which specifically recognizes a nucleic acid sequence in the original Anchor Oligonukleotid, of which at the matrix remaining Anchor Oligonukleotid are separated. The aufligierte nucleic acid sequence < RTI ID=13.5> hängt< /RTI> now at the Splinker Oligonukleotid anligierten last. The elongated Splinker Oligonukleotid is transferred after inactivation of the restriction enzyme from the original reaction beginning into a new reaction container and < there with a aufligierten Anchor Oligonukleotid; RTI ID=13.6> linked, < /RTI> the restriction enzyme specific with a Splinker Oligonukleotid cut was < RTI ID=13.7> (I.< /RTI> Transposition). The person skilled in the art it is evidently that it can concern with the aufligierten nucleic acid sequences freely selectable sequences, both < RTI ID=13.8> unterschiedlich< /RTI> and to be identical can. From < RTI ID=13.9> 1.< /RTI> Transposition resulting Ligationsprodukt is cut again with a Anchor specific Restriktionsendonuklease and ligiert again with an analog received aufligierten Anchor Oligonukleotid (2. Transposition). In this way the length of the aufligierten nucleic acid sequences doubles itself then with each further step. < RTI ID=13.10> Verknüpfung< /RTI> the DNA fragments < effected in each case; RTI ID=13.11> über< /RTI> complementary overhangs, is however otherwise perfectly < RTI ID=13.12> sequenzunabhängig.< /RTI> The only restriction thereby is that Anchor and the Splinkerspezifi restriction interfaces in the sequence which can be synthesized do not occur < RTI ID=13.13> may, < /RTI> because otherwise the DNA to be cut also internally < RTI ID=13.14> würde.< /RTI> < RTI ID=13.15> Jeweils< /RTI> before a cracking at a Anchor specific restriction interface and the Transposition following on it fakultativ a Exonukleaseschritt can be introduced, around those Transposition < RTI ID=13.16> vollständig< /RTI> aufligierter Splinker Oligonukleotide too < RTI ID=13.17> verhindern.< /RTI> < RTI ID=13.18> für< /RTI> the method necessary sequence-specific cracking know instead of by TypIIS Restriktionsendonukleasen in Principle also by analog < RTI ID=14.1> funktionierende< /RTI> Ribozyme take place.

On the basis of 20 pair of cousins is enough for sequence (which one < for Splinker with 4 a NT; RTI ID=14.2> Überhang< /RTI> by 5 gradual Ligationen that for this < RTI ID=14.3> benötigten< /RTI> Origin Splinker from the library received can), leaves themselves < by only 7 further; RTI ID=14.4> Ligationsschritte< /RTI> one < RTI ID=14.5> doppelsträngige< /RTI> DNA sequence of 2560 pairs of cousins length < RTI ID=14.6> synthetisieren.< /RTI> With cycle times of approx. < RTI ID=14.7> 1< /RTI> Hour can be synthesized any DNA sequence of this length within 12 hours. The expenditure of time can be halved by an optimization of the reaction conditions on approximately 6 hours.

With 4 nucleotides long overhangs a library is < by 65536 different Splinker Oligunukleotiden; RTI ID=14.8> needed, < /RTI> around all < RTI ID=14.9> möglichen< /RTI> Nucleic acid sequences manufacture too < RTI ID=14.10> können.< /RTI>

This number results from the following computation: there are 256 < RTI ID=14.11> mögliche< /RTI> 4 nucleotides long overhangs (44 = 256), likewise many sequence variants exist for the four directly bordering nucleotides, which form the overhang with the next Ligationsschritt. Altogether from it a total number of < results; RTI ID=14.12> 44< /RTI> times 44 = < RTI ID=14.13> 48< /RTI> = 65536 Splinker Oligonukleotide, with which < RTI ID=14.14> sämtliche< /RTI> < RTI ID=14.15> möglichen< /RTI> Sequence variants to be represented < RTI ID=14.16> können.< /RTI> With 3 reduces nucleotides long overhangs < RTI ID=14.17> Komplexität< /RTI> < RTI ID=14.18> benötigten< /RTI> Splinkerbibliothek on < accordingly; RTI ID=14.19> 43 times 43 =< /RTI> 4096, with two nucleotides long overhangs on 42 times < RTI ID=14.20> 42< /RTI> = 256, with 5 nucleotides are enough for overhangs sichauf45mal45=1048576erhöhen. Condition for this they building block principle is the presence of a complete Splinkerbibliothek (for 2 NT of overhangs 256 Oligonukleotide, for 3 NT of overhangs 4096 Oligonukleotide, for 4 NT of overhangs 65536 Oligonukleotide, for 5 NT of overhangs 1048576 Oligonukleotide) as well as a Anchorbibliothek (4,
16,64,256 or 1024 Oligonukleotide with < RTI ID=14.21> 1,2,3,4< /RTI> or 5 NT < RTI ID=14.22> Overhangs).
< /RTI> The latter is not < however necessarily; RTI ID=14.23> necessarily, < /RTI> since the different overhang sequences just as well by an upstream Ligationsschritt with suitable Splinker Oligonukleotiden to be produced < RTI ID=14.24> können.< /RTI>

In principle all single steps are < RTI ID=14.25> erfindungsgemässen< /RTI> Method automizable, so that the making of whole genes is as simple as the synthesis of Oligonukleotiden. Besides < RTI ID=14.26> eröffnet< /RTI> by < itself; RTI ID=14.27> erfindungsgemässe< /RTI> Method a reduction of costs potential in < RTI ID=14.28> beträchtlicher< /RTI> < RTI ID=14.29> Höhe.< /RTI>

First of all all can < RTI ID=14.30> benötigten< /RTI> Enzymes to be industrially manufactured. < RTI ID=14.31> Zweitens< /RTI> can < RTI ID=15.1> Investitionen< /RTI> for < RTI ID=15.2> Splinkerlibrary< /RTI> to be lowered clearly, as the individual Splinker Oligonukleotide up to the last 4 nucleotides < RTI ID=15.3> 5 ' - Überhangs< /RTI> EN bloc to be synthesized.

The synthesis reaction is then portioniert into 4 equal parts; the four different nucleotides are then < in separate reactions to; RTI ID=15.4> nächsten< /RTI> (in the final product fourth from last) position < RTI ID=15.5> angehängt.< /RTI> Afterwards the four single reactions are again < RTI ID=15.6> quartered, < /RTI> according to which the third last nucleotide < RTI ID=15.7> angehängt< /RTI> becomes etc. Instead of 65536 single syntheses < RTI ID=15.8> würde< /RTI> one then only 256 syntheses in an accordingly larger and therefore more favorable ruler < RTI ID=15.9> benötigen.< /RTI> Furthermore the 256 can < RTI ID=15.10> möglichen< /RTI> 4 nucleotides are enough overhangs by one " blunt end ligation " at 256 different Anchor Oligonukleotide, following Exonukleasebehandlung, washing and finally for restriction with < RTI ID=15.11> Anclior specific Restriktionsendonuklease< /RTI> are produced.

In this way the 65536 could < RTI ID=15.12> benötigten< /RTI> Splinker Oligonukleotide to be economically manufactured. Furthermore a complex Aufreinigung of all 65536 Splinker Oligonukleotide could be gone around in this way, since not-reactive false sequences by this method < RTI ID=15.13> entfernt< /RTI> become. Since an extremely high purity of the assigned Oligonukleotide essentially < RTI ID=15.14> für< /RTI> the success of faultless syntheses is < RTI ID=15.15> müssen< /RTI> this to be pre-treated anyway accordingly. Besides one must practically < RTI ID=15.16> vollständige< /RTI> Absence of Exonukleasen during Restriktions-und Ligationsschritte < RTI ID=15.17> gewährleistet< /RTI> its, thus < RTI ID=15.18> those overhang < /RTI> sequences intact remain <, for the following Ligationen; RTI ID=15.19> benötigt< /RTI> become. Above all if < RTI ID=15.20> Exonuklease Zwischenschritte< /RTI> to < RTI ID=15.21> Entfernung< /RTI> nichtligierter Anchor Oligonukleotide to be used, < RTI ID=15.22> müssen< /RTI> this Exonukleasen < RTI ID=15.23> gründlich< /RTI> away-washed < RTI ID=15.24> und/oder< /RTI> are inactivated.

Anchor and the Splinker Oligonukleotide can be built up both in each case from a complementary single strand and from two complementary Plus-und each minus strands. The nucleic acid sequences do not have < RTI ID=15.25> vollständig< /RTI> complementary its; die selbstkomplementären Einzelstrang-Oligonukleotide können einen Loop aufweisen und die komplementären Plus-und <RTI ID=15.26>Minus-Stränge</RTI> can only partial ones be more complementary. With Anchor and Splinker Oligonukleotiden, those from ever two complementary Plus-und Minus< RTI ID=15.27> Strängen< /RTI> compound, (i) the fusing temperature of the Doppelstranghybrids is sufficient must be highly, around one < RTI ID=15.28> Denaturierung< /RTI> the compound Anchor and Splinker Oligonukleotide and possibly an unintentional transfer of the single strands not coupled resulting from it to a solid phase too < RTI ID=15.29> verhindern< /RTI> and < RTI ID=15.30> müssen< /RTI> < RTI ID=15.31> (ii) < /RTI> those not in each case to extending ends by suitable modifications blocked its. Oligonukleotide from two complementary Plus-und minus strands possess opposite Oligonukleotiden from a complementary < RTI ID=16.1> Einzelstrang< /RTI> determined advantages. Selfcomplementary (Snap bake) Oligonukleotide causes with the Aufreinigung often < RTI ID=16.2> gewisse< /RTI> Difficulties, since they have a tendency in high concentration for the formation of networks. < RTI ID=16.3> Einzelsträngige< /RTI> Teiloligonukleotide are to be won also shorter and thus at smaller expenditure in higher purity. < RTI ID=16.4> Für< /RTI> determined < RTI ID=16.5> Ausführungsformen< according to invention; /RTI> from two Teiloligonukleotiden built up (?bi-partite?) Anchor Oligonukleotide is used.

In particularly preferential embodiments the Anchor contains and/or. Splinker the following combinations at recognition sequences: Anchor Splinker CGTCTCN^NNNN < RTI ID=16.6> (Esp3I, < /RTI> BsmBI) < RTI ID=16.7> GGTCTCNANNNN~< /RTI> (BsaI, < RTI ID=16.8> Eco31I,) < /RTI> < RTI ID=16.9> GGTCTCNNNNN (Bsal, Eco311,?) CGTCTCNNNNN (Esp31, BsmBI) GAAGACNN^NNNN (BbsI, BpiI?) ACCTGCNNNN^NNNN~ (BspMI, Acc36I) < /RTI> < RTI ID=16.10> ACCTGCNNNN^NNNN< /RTI> < RTI ID=16.11> (BspMI, < /RTI> Acc36I) < RTI ID=16.12> GAAGACNN^NNNN (BbsI, BpiI?)< /RTI> < RTI ID=16.13> GCAGTG~NN^ (BtsI) GCAATG~NN^ (BsrDI, Bse3DI,) GCAATG~NN^ (BsrDI, Bse3DI,) GCAGTG~NN^ (BtsI) < /RTI> BfuI) ACTGGGNNNN~N^ (BfiI, BmrI) GTATCCNNNNN~N^ (BciVI, < RTI ID=16.14> ACTGGGNNNN N^ (BfiI, BmrI) GTATCCNNNNN N^ (BciVI, BfuI) < /RTI> A further aspect of the present invention is < a kit to the making after nucleic acid; RTI ID=16.15> erfindungsgemässen< /RTI> Method. The kit can from a library of all necessary Anchor and Splinker Oligonukleotide, furthermore a solid phase, to which the Anchor Oligonukleotide can be coupled, preferably magnetized Beads, suitable reaction containers, ligase, if necessary one < RTI ID=16.16> Topoisomerase< /RTI> and/or a 3 ' - 5 ' Exonuklease and/or Phosphatase, at least two different TypII Restriktionsendonukleasen, which cut outside of their recognition sequence, as well as all < RTI ID=16.17> benötigten< /RTI> Reaction buffers exist. Preferentially is < RTI ID=16.18> fernerhin< /RTI> a pipetting station with a kiihlbaren sample storage vessel with an appropriate software control, which < RTI ID=16.19> sämtliche< /RTI> Steps < RTI ID=16.20> erfindungsgemässen< /RTI> Method automatically < RTI ID=16.21> durchfiihrt< /RTI> The present invention permits a complete automation of the entire gene synthesis process by the supply of a library again and again more using, at least partly < RTI ID=17.1> doppelsträngiger< /RTI> highly pure Oligonukleotide with recognition sequences < RTI ID=17.2> für< /RTI> determined TypIIS Restriktionsendonukleasen (so-called " Outside Cutter "). Furthermore automation permitted by the supply of a method, which < the parallel synthesis of gene fragments and their sequence-independent; RTI ID=17.3> Verknüpfung< /RTI> as well as in any place permits due to one < RTI ID=17.4> orientierungsgebundenen< /RTI> Extension < RTI ID=17.5> Startmoleküle< /RTI> < RTI ID=17.6> über< /RTI> deren Bindung an eine Festphase (die nicht zu ligierenden Enden sind durch geeignete Modifikationen bzw. Loop sequences blocks) and a defined set of recursive procedures (Ligations, Wasch-und < RTI ID=17.7> Restriction steps), < /RTI> by a robot to be processed can.

Below certain aspects of the present invention are exemplarily shown, those on the complete synthesis of whole genes

by are < RTI ID=17.8> erfindungsgemässe< /RTI> Methods are based.

< RTI ID=17.9> 1.< /RTI> Preparation of one < RTI ID=17.10> cDNA, < /RTI> if only the protein sequence admits is it comes < RTI ID=17.11> häufig< /RTI> forwards that only the amino acid sequence or parts of the amino acid sequence of a protein admits is, however not it < RTI ID=17.12> cDNA< /RTI> or genomische sequence. Ways of degeneration of the genetic code it is usually < RTI ID=17.13> possible, < /RTI> the appropriate gene < directly; RTI ID=17.14> über< /RTI> to amplifizieren a PCR of a suitable cDNA bank. One looks for therefore regions, in those amino acids such as tryptophan, methionine and/or. Asparagine, Aspartat, Glutamat, glutamine, tyrosine, phenylalanine, cysteine or lysine < RTI ID=17.15> gehäuft< /RTI> arise, since it < RTI ID=17.16> für< /RTI> these amino acids only and/or. two Codons gives. < RTI ID=17.17> Sofern< /RTI> succeeds, with < RTI ID=17.18> niedrig< /RTI> degenerated < RTI ID=17.19> Primern< /RTI> a PCR fragment of the expected value to receive, is used this as sonde, around which < RTI ID=17.20> dazugehörige< /RTI> Gen aus einer cDNA-Bank zu klonieren. This work is < nowadays by the availability by gene arrays and clone collections in many; RTI ID=17.21> Fällen< /RTI> substantially facilitates, but to the one such auxiliary means stand only < RTI ID=17.22> für< /RTI> a limited number from organisms and cell types to < RTI ID=17.23> Order, < /RTI> on the other hand is even < when being present the complete; RTI ID=17.24> cDNA< /RTI> usually still one < RTI ID=17.25> Umklonierung< /RTI> into a suitable express ion vector necessarily. The expenditure of time can depending upon difficulty of the pro jet with until two weeks, in < RTI ID=17.26> Extremfällen< /RTI> but quite also at several months to years are appropriate. With < RTI ID=17.27> erfindungsgemässen< /RTI> Method can on the basis of a well-known protein sequence < RTI ID=17.28> Expressionskonstrukt< /RTI> with one < RTI ID=17.29> für< /RTI> the desired organism optimized Codon Usage in to two days to be manufactured. < RTI ID=17.30> For this muss< /RTI> the organism, in which the protein natural-proves is exprimiert, not at all to be available, since the DNA sequence from the well-known protein sequence < RTI ID=18.1> abgeleitet< /RTI> will can, without a Template must be present. With improved Proteinsequenzierungsmethoden it is in the future < RTI ID=18.2> möglich< /RTI> its, proteins with interesting < RTI ID=18.3> Enzymaktivitäten< /RTI> from arbitrary organisms to sequenzieren and without the detour < RTI ID=18.4> über< /RTI> < RTI ID=18.5> CDNA Klonierung< /RTI> mittels des <RTI ID=18.6>erfindungsgemässen</RTI> Method directly into each desired express ion system too < RTI ID=18.7> überführen.< /RTI>

2. Making of designer genes and designer proteins further aspect of the present invention is the simple preparation of designer genes and/or. Designer proteins, D. h. the coupling functional domains of different proteins, in order to manufacture for example enzymes with new or changed properties. With knowledge < RTI ID=18.8> Röntgenkristallstruktur< /RTI> a protein can then very < RTI ID=18.9> gezielte< /RTI> Changes such as z. B. the insertion < RTI ID=18.10> definierter< /RTI> Left domains or a Redesign of a connection bag to be made, around new functions or changed < RTI ID=18.11> Spezifitäten< /RTI> into proteins < RTI ID=18.12> einzuführen.< /RTI> By purposeful < RTI ID=18.13> Proteindesign< /RTI> one can design for example adjustable catalytic centers, which are activated by a Konformationsänderung of the protein due to the connection of a specific ligand. In this way designer proteins can be manufactured, the z. B. on the connection of a certain virus protein one < RTI ID=18.14> Caspase Aktivität< /RTI> unfold, which releases Apoptose then in infected cells. First versions of such highly specific medicines were already described; see. Vocero Akbani A. M., Heyden N. V., Lissy N. A., Ratner L., Dowdy S. F., Nat Med, 1999 January, 5: 1,29-33. Further proteins can thereby < RTI ID=18.15> stabilisiert< /RTI> that at certain positions amino acids are inserted, those are < RTI ID=18.16> zusätzliche< /RTI> < RTI ID=18.17> Salzbrücken< /RTI> form < RTI ID=18.18> können.< /RTI> Thus the tolerance can be improved in relation to high temperatures, which < among other things; RTI ID=18.19> für< /RTI> < RTI ID=18.20> Waschmittelindustrie< /RTI> is favourable.

< RTI ID=18.21> Sofern< /RTI> is <, can by the point-exact expression a desired enzymatic admits the cathedral to structures of certain functional regions; RTI ID=18.22> Aktivität< /RTI> from an unwanted to be separated. Likewise multi-enzyme complexes can be designed, which catalyze a whole number of different reactions < RTI ID=18.23> können.< /RTI> Thus leave themselves < RTI ID=18.24> Verbesserungen< /RTI> with the synthesis of many organic compounds or some syntheses reach even only < RTI ID=18.25> ermöglichen.< /RTI> This < RTI ID=18.26> eröffnet< /RTI> completely new perspectives, since many organic syntheses, with which today still environmentally hazardous < RTI ID=18.27> Lösungsmittel< /RTI> and catalysts to be used < RTI ID=18.28> must, < /RTI> in the future by such custom-made biocatalysts to be replaced < RTI ID=18.29> können.< /RTI>

3. Systematic Mutagenese as replacement < RTI ID=19.1> für< /RTI> randomized Mutagenisierung one < RTI ID=19.2> häufig< /RTI> occurring task in < RTI ID=19.3> biochemisch< /RTI> < RTI ID=19.4> orientierten< /RTI> < RTI ID=19.5> Molekularbiologie< /RTI> consists of picking out from many protein variants diej some which < RTI ID=19.6> höchste< /RTI> enzymatic < RTI ID=19.7> Aktivität< /RTI> or < RTI ID=19.8> stärkste< /RTI> Connection to substrate or another protein exhibits. One proceeds then usually in such a way that one introduces a set of coincidental mutations of one or more amino acids and the developing variants to a suitable Screening method analyzed. It is also in principle < RTI ID=19.9> possible, < /RTI> all mutants to manufacture separately, however this is accomplished rarely from Zeit-und cost reasons. With a randomized < RTI ID=19.10> Mutagenisierung< /RTI> control is < RTI ID=19.11> über< /RTI> the developing mutants naturally much limits, to amino acid substitutions determined there process-determined < RTI ID=19.12> häufiger< /RTI> are found as others, on the other hand, since it hardly < itself; RTI ID=19.13> vermeiden< /RTI> < RTI ID=19.14> leaves < /RTI> that with this method also < RTI ID=19.15> Stopcodons< /RTI> are introduced. With < RTI ID=19.16> erfindungsgemässen< /RTI> Methods can be represented however all desired mutants purposeful and without large expenditure and as proteins < RTI ID=19.17> exprimieren.< /RTI>

4. Preparation of synthetic genes, in particular to the inset as DNA Vakzine in many < RTI ID=19.18> Fällen< /RTI> it is desirable to optimize the Proteinexpression of certain genes in heterolied systems. This can by the use of strong promoters very < RTI ID=19.19> häufig< /RTI> to be only partially reached. Depending on, which < organism; RTI ID=19.20> für< /RTI> the expression used is <, knows itself the use of certain Codons; RTI ID=19.21> für< /RTI> a amino acid on the attainable Genexpression affect favourably or disadvantageful. Like that for example many retrovirale gene products are in eukaryotischen cells only badly translatierbar, since they are usually much RK realm and in < RTI ID=19.22> höheren< /RTI> Eukaryonten seltene Codons benutzen. Particular one < RTI ID=19.23> für< /RTI> the inset of such gene sequences as DNA Vakzine it therefore is of great advantage if their Codongebrauch for mammalian

cells is optimized. In the same way certain RNA structures can to one < RTI ID=19.24> Instabilität< /RTI> the Transkripte lead, which can affect the Genexpression likewise negative.

Such elements can by Codonveränderungen with < RTI ID=19.25> erfindungsgemässen< /RTI> Method to be likewise easily switched off.

5. Analysis of protein domains through < RTI ID=19.26> Deletions oder< /RTI> Punktmutagenese the analysis of mutants is very often the composition of the choice with the functional characterisation of proteins. Existed both < RTI ID=19.27> für< /RTI> the preparation of < RTI ID=19.28> Deletions wie< /RTI> also Punktmutanten a number of established methods, however are usually very time-consuming and labor intensive these. Deletionen are usually manufactured by insertion by left sequences or by a PCR with primer, whose ends are more complementary to different partial sequences. In order to receive a whole series of defined Deletionen, is < RTI ID=20.1> häufig< /RTI> a two-step procedure necessarily, with which first certain restriction interfaces are introduced, over which then the desired Deletionen < RTI ID=20.2> eingeführt< /RTI> are < RTI ID=20.3> können.< /RTI> With accordingly < RTI ID=20.4> konzipierten< /RTI> Primers and a Mehrfragmentligation such Deletionen can be manufactured also in principle in a step, however the chances of success are rather small thereby. In all these < RTI ID=20.5> Fällen< /RTI> the Wildtyp DNA must be present as Template, which < with; RTI ID=20.6> erfindungsgemässen< /RTI> Method is not necessary. < RTI ID=20.7> Darüber< /RTI> outside Deletionsmutanten can be manufactured, since it is not at all necessary to introduce restriction interfaces for which one only suitable places find must, so that < RTI ID=20.8> eingeführten< /RTI> Mutations changes in the protein sequence do not cause (so-called " silent site " mutations). Also the preparation of double or Tripletmutanten is < with; RTI ID=20.9> erfindungsgemässen< /RTI> Method < RTI ID=20.10> möglich.< /RTI> < RTI ID=20.11> Für< /RTI> functional mapping of a protein mutations simultaneous also restriction interfaces know < in its gene sequence by means of mentioned " silent site "; RTI ID=20.12> für< /RTI> a large number of different Restriktionsendonukleasen < RTI ID=20.13> eingeführt< /RTI> become, with whose aid arbitrary Deletionen can be manufactured. In many < RTI ID=20.14> Fällen< /RTI> wird daher die klassische Mutationsanalyse verzichtbar und kann durch das schnellere und genauere <RTI ID=20.15>erfindungsgemässe</RTI> Method to be replaced.

6. Coupled one in vitro < RTI ID=20.16> Transkriptions/Translationssysteme< /RTI> < RTI ID=20.17> (?EasyProTM?) < /RTI> Coupled ones in vitro Transkriptions/lateral adjustment systems are used to the quick synthesis by proteins in the analytic ruler, z. B. for connection studies or Kopräzipitationsassays. < RTI ID=20.18> Hierfür< /RTI> to exprimierenden sequences into a vector are kloniert, which < a Promoter; RTI ID=20.19> für< /RTI> eine RNA-Polymerase <RTI ID=20.20>enthält.</RTI> Mit Hilfe dieser Polymerase wird mRNA transkribiert, die in einem RNA-depletierten <RTI ID=20.21>Weizenkeim-oder</RTI> Retikulozytenextrakt in das gewünschte Protein translatiert wird, das aufgrund der geringen Ausbeute und der einfacheren Nachweisbarkeit meist mit <RTI ID=20.22>35S-Methionin</RTI> oder Cystein radioaktiv markiert ist. A still faster alternative is < on; RTI ID=20.23> erfindungsgemässen< /RTI> Verfahren basierende <RTI ID=20.24>EasyProTM-System.</RTI> In einem Anchor-Oligonukleotid, welches einen T7 (SP6) Promoter, eine interne Ribosomenbindungsstelle sowie ein Hexahistidin-Tag <RTI ID=20.25>enthält,</RTI> through one < RTI ID=20.26> Restriktion< /RTI> with XcmI < individually; RTI ID=21.1> Thymidinüberhang< /RTI> produced, which can be ligiert directly with a PCR product. Three < RTI ID=21.2> EasyProTM Anchor Oligonukleotide< /RTI> with different reader asters, in order all are sufficient in correct orientation ligierten PCR fragments to translatieren. Mittels terminaler Transferase oder durch Ligation eines entsprechenden Splinker-Oligonukleotids an das <RTI ID=21.3>3'-Ende</RTI> des PCR-Produkts kann man zudem leicht einen künstlichen poly-A-Schwanz in das DNA-Template <RTI ID=21.4>einführen,</RTI> which the RNA Transkript stabilized and thus < RTI ID=21.5> für< /RTI> eine <RTI ID=21.6>höhere</RTI> Translationseffizienz sorgt. Furthermore the DNA sequences coding for the desired protein can after cracking with a Restriktionsendonuklease also directly to a modified < RTI ID=21.7> EasyProTM Anchor< /RTI> with suitable 4 NT a long overhang to be ligiert.

Ein weiterer Aspekt der vorliegenden Erfindung ist die Bereitstellung eines Minireaktors zur schnellen Synthese von Proteinen. In der unteren Reaktionskammer des Minireaktors findet die Transkription der an Streptavidin-beschichtete Beads gekoppelten Expressions-Anchor Nukleinsäuresequenz statt. With it the developing mRNAs are < RTI ID=21.8> über< /RTI> ihren 3'-poly-A Schwanz an Oligo-dT gekoppelte Beads gebunden, welche sich ebenfalls in der unteren Reaktionskammer befinden. Dort <RTI ID=21.9>läuft</RTI> also the translation mRNAs in a reticulocyte excerpt off. Diese Kammer wird durch eine Ultrafiltrationsmembran mit einem MWCO (Molekulargewichtsausschluss) von ca. 200 kD von einer <RTI ID=21.10>darüber</RTI> lying second chamber separated. This contains Beads, which < the protein of; RTI ID=21.11> Interesse< /RTI> to bind can (z. B.

<RTI ID=21.12>Ni +-NTA-Beads für</RTI> Proteine mit einem Hexahistidin-Tag). Durch eine kontinuierliche Zufuhr von Pufferlösung mit frischen <RTI ID=21.13>niedermolekularen</RTI> Reactants (Aminoacyl tRNAs, Ribonukleotidtriphosphate, CAP analogue and Creatinphosphat) the production is < RTI ID=21.14> über< /RTI> <RTI ID=21.15>längere</RTI> Time away maintain. Simultaneous one is < thereby; RTI ID=21.16> synthetisierte< /RTI> Protein from the lower into the upper reactor < RTI ID=21.17> printed, < /RTI> where it at the Beads < RTI ID=21.18> hängen< /RTI> remains.

Alternativ kann diese Kammer durch eine weitere Ultrafiltrationsmembran abgeschlossen werden, deren Ausschluss so gewählt ist, dass sie <RTI ID=21.19>für</RTI> Puffer und kleinere <RTI ID=21.20>Moleküle,</RTI> not however < RTI ID=21.21> für< /RTI> the desired protein permeabel is. This collects therefore in the upper chamber and can from there in up-cleaned form insulated become. The yields attainable thereby are not < only; RTI ID=21.22> für< /RTI> most analytic experiments sufficiently, but can replace even protein express ion experiments in the small ruler. If it concerns for example, the specific enzymatic < RTI ID=21.23> Aktivität< /RTI> more differently < RTI ID=21.24> Proteinmutanten< /RTI> to determine, these had < RTI ID=21.25> hierfür< /RTI> so far in < RTI ID=21.26> aufwendigen< /RTI> Preliminary tests kloniert, exprimiert and < RTI ID=21.27> aufgereinigt< /RTI> become. Since practically everything these steps in < RTI ID=22.1> erfindungsgemässen< /RTI> < RTI ID=22.2> EasyProTM Verfahren< /RTI> are already integrated, thereby more substantially one < RTI ID=22.3> Zeitvorteil< /RTI> opposite konventiellen methods reaches.

With a modification of the managing described < RTI ID=22.4> erfindungsgemässen< /RTI> Method can be

manufactured simply and economically Peptidbibliotheken, which < among other things to the Epitopmapping of; RTI ID=22.5> Antikörpern< /RTI> or to the identifying of immunogen Epitope in proteins of viruses, bacteria or funguses < RTI ID=22.6> benötigt< /RTI> become (to the quick establishment of serologischer proof systems). < RTI ID=22.7> Hierfür< /RTI> modified are < RTI ID=22.8> EasyProTM Anchor Oligonukleotide< /RTI> gradually by Splinkerligationen < RTI ID=22.9> elongated, < /RTI> so that < RTI ID=22.10> für< /RTI> the desired peptides coding sequences develop. In the last step a prefabricated Endsplinker is anligiert, which < RTI ID=22.11> für< /RTI> a C-terminal day, < RTI ID=22.12> Stopcodon< /RTI> as well as a Poly A tail codes. The Ligationsprodukte is transliterated and translatiert in the described mini reactor. The finished peptides become after termination of the translation and several wash steps with a specific protease, z. B. Enterokinase or factor Xa, at which of < RTI ID=22.13> EasyProTM Anchor < /RTI> Oligonukleotid coded interface abgespalten and from the upper reactor washed. With the help of C-terminal tags these can to a solid phase < RTI ID=22.14> für< /RTI> following tests to be bound. The peptides are present besides already in up-cleaned form and can directly < RTI ID=22.15> für< /RTI> following applications to be used. Since the same Anchor Oligonukleotid is used in each case and < RTI ID=22.16> benötigten< /RTI> Splinker Oligonukleotide from prefabricated an already < RTI ID=22.17> Teilstück< /RTI> in few steps to be aufligiert, are smaller the resulting costs can than with a conventional Peptidsynthese.

7. Preparation of Ribozymen or Aptameren analog one to the protein synthesis described above leaves themselves Promoter to Anchor Oligonukleotide with a T7 (SP6) also to the preparation and < RTI ID=22.18> Mutagenisierung< /RTI> use from RNAs. The system is suitable in particular for the synthesis of different Ribozyme, since the DNA sequences on an elongated Splinker Oligonukleotid, coding for the Ribozyme, can be anligiert to an activator module on a Anchor Oligonukleotid. Above all can exactly defined < RTI ID=22.19> Ribozymtemplatebibliotheken< /RTI> are provided, which can be amplifizieren by PCR easily. With < RTI ID=22.20> erfindungsgemässen< /RTI> Methods can < RTI ID=22.21> Ribozymtemplatesequenzen< /RTI> on the nucleotide to be manufactured exactly, without < RTI ID=22.22> hierfür< /RTI> Klonierungsarbeiten are necessary. By introduction of link sequences one can manufacture Ribozyme, which < itself; RTI ID=23.1> über< /RTI> a DNA/RNA to any chemical connection such as peptides, complementary to the link sequence, < RTI ID=23.2> Nucleic acids, < /RTI> aliphatische Kohlenwasserstoffe, Ester, Ether oder Alkohole koppeln lassen. If this connection is present bound to a solid phase, Ribozyme leave themselves < RTI ID=23.3> selektionieren, < /RTI> this connection split. < RTI ID=23.4> Diejenigen< /RTI> Ribozyme, which have itself from the connection to the solid phase " released ", can by reverse Transkription and following asymmetric PCR in < RTI ID=23.5> einzelsträngige< /RTI> < RTI ID=23.6> DNA Moleküle< /RTI> are transferred. These are then < RTI ID=23.7> über< /RTI> the link sequence to an accordingly modified Anchor Oligonukleotid hybridizes and ligiert. The used Anchor Oligonukleotid is in such a way designed that it < a T7-Promoter; RTI ID=23.8> contains, < /RTI> over with the help of the T7-Polymerase again the Ribozym to be received can. By the use of an inaccurate Reversen Transkriptase (z. B. HEAVE blank) leave themselves < RTI ID=23.9> zufällige< /RTI> Mutations < RTI ID=23.10> einführen.< /RTI> The selection pressure can by ever shorter Inkubationen < RTI ID=23.11> erhöht< /RTI> become, so that präferentiell Ribozyme with a high Akti< RTI ID=23.12> vität< /RTI> are amplifiziert. Analog ones leave themselves according to the same principle also to Ribozyme with < RTI ID=23.13> Fähigkeit< /RTI> selektionieren, eine Bindung zur festen Phase zu <RTI ID=23.14>vermitteln.</RTI>

8. Use of with < RTI ID=23.15> erfindungsgemässen< /RTI> Methods produced ssDNAs in the diagnosis < RTI ID=23.16> (PathoCheckT) < /RTI> With the diagnosis of < RTI ID=23.17> Infektionskrankheiten< /RTI> usually one < RTI ID=23.18> häufig< /RTI> < more directly; RTI ID=23.19> Erregernachweis< /RTI> z. B. by PCR requires. Particularly in the Transfusionsmedizin is important it to recognize and segregate contaminated blood samples surely. < RTI ID=23.20> hierfür< /RTI> <RTI ID=23.21>üblicherweise</RTI> assigned serologischen Assays can do this only then < RTI ID=23.22> ensure, < /RTI> if the infection of the donor already some time < RTI ID=23.23> is past, < /RTI> so that already < RTI ID=23.24> Antikörper< /RTI> formed are.

While a window of up to 12 weeks (< in; RTI ID=23.25> Extremfällen< /RTI> auch <RTI ID=23.26>länger)</RTI> for example still none are < with the HIV infection; RTI ID=23.27> Antikörper< /RTI> im Blut <RTI ID=23.28>nachweisbar,</RTI> although already a solid Virusreplikation takes place. Since a routine < RTI ID=23.29> PCR Untersuchung< /RTI> all samples from cost reasons, becomes this is in many places hardly feasible (if < RTI ID=23.30> at all) < /RTI> an Pools von Einzelspenden <RTI ID=23.31>durchgeführt.</RTI> The problem thereby is that thereby the actually very high < RTI ID=23.32> Sensitivität< /RTI> sinks, since the quantity < RTI ID=23.33> für< /RTI> the analysis of assigned material does not < RTI ID=23.34> beliebig< /RTI> < RTI ID=23.35> erhöht< /RTI> will can. Bei Viruserkrankungen wie HIV, bei denen ein <RTI ID=23.36>Grossteil</RTI> the viruses extracellularly, this is present by can become still to some extent balanced a concentrating of the viruses by Zentrifugation, with predominantly < RTI ID=23.37> zellassoziierten< /RTI> Viruses < RTI ID=23.38> funktioniert< /RTI> however usually less well. Man kann zwar zunächst DNA oder RNA aus den Blutzellen <RTI ID=24.1>isolieren,</RTI> jedoch nur einen Bruchteil davon in der PCR-Reaktion einsetzen, da sonst unspezifische PCR-Produkte überhandnehmen. Daher muss in solchen <RTI ID=24.2>Fällen</RTI> eine Vorselektion des zu amplifizierenden Materials durchgeführt werden. For this one < RTI ID=24.3> einzelsträngiges, < /RTI> mit dem <RTI ID=24.4>erfindungsgemässen</RTI> Verfahren hergestelltes Produkt eingesetzt, das mit einem modifizierten Anchor-Oligonukleotid hergestellt wird. In diesem Fall verwendet man ein Anchor-Oligonukleotid aus zwei getrennten komplementären <RTI ID=24.5>Strängen,</RTI> of those at 5 ' - the end modified, z. B. biotinyliert, is, which others at the 3 ' - end is blocked. Nach der Synthese der Virus-Sequenz wird der nichtbiotinylierte Strang durch Waschen mit einer denaturierenden Lösung abgetrennt, so dass eine <RTI ID=24.6>einzelsträngige</RTI> Antisense-DNA verbleibt. This can do 5 with that ' - part Anchor Oligonukleotid and one biotinylierten < RTI ID=24.7> endständigen< /RTI> Oligonukleotid amplifiziert werden, <RTI ID=24.8>sofern</RTI> more materials < RTI ID=24.9> benötigt< /RTI> becomes. From this PCR product only one strand is biotinyliert, which can do others by denaturing is separated. This Antisense DNA can be used now in order to enrich virale RNAs or DNAs from a complex mixture as a Zelllysat or a nucleic acid preparation, by hybridizing these with one another, the hybrid to a Streptavidin coated < RTI ID=24.10> Träger< /RTI> (Support) binds and non-hybridized components under stringer ducks conditions < RTI ID=24.11> wegwäscht.< /RTI> In einem zweiten Schritt können die <RTI ID=24.12>angereicherten</RTI> RNAs oder DNAs dann mit einer konventionellen PCR mit <RTI ID=24.13>Primern</RTI> aus dem nicht-hybridisierten Teil der RNA oder DNA amplifiziert und nachgewiesen werden. Dies kann üblicherweise über Gelelektrophorese der Produkte <RTI

ID=24.14>geschehen</RTI> oder durch Fluoreszenzanalyse oder durch einen nachgeschalteten ELISA, unter der Voraussetzung, dass ein entsprechend modifizierter Primer verwendet wurde. Advantages < RTI ID=24.15> erfindungsgemässen< /RTI> Method it are that nearly < RTI ID=24.16> beliebig< /RTI> hohe Mengen an Ausgangsmaterial eingesetzt werden können, was die <RTI ID=24.17>Sensitivität</RTI> der Analyse verbessert, dass auch mehrere Targets gleichzeitig untersucht und bei Verwendung verschieden fluoreszenzmarkierter Primer auch differenziert werden können und dass es <RTI ID=24.18>für</RTI> arbitrary Pathogene such as bacteria, funguses or viruses is applicable. Durch die Voranreicherung der zu amplifizierenden Sequenzen werden <RTI ID=24.19>nebenbei</RTI> also clearly reduces background problems. Bei entsprechender <RTI ID=24.20>Miniaturisierung</RTI> kann eine <RTI ID=24.21>grosse</RTI> Number of different Pathogene simultaneous on a chip to be tested, whereby the analysis costs are extremely lowered < RTI ID=24.22> können.< /RTI>

9. ?< genes Profiling?; RTI ID=24.23> (GProT) < /RTI> In der molekularbiologischen Forschung und zunehmend auch in der molekularen Diagnostik wird die Expression bestimmter Gene auf der RNA-Ebene quantitativ untersucht. Standard devices for this are the Northern Blot, the S1-Mapping or the " ribonuclease Protection Assay " (RPA), usually in connection with < RTI ID=25.1> radioaktiv< /RTI> markierten Sonden. Die vorstehend beschriebenen <RTI ID=25.2>einzelsträngigen</RTI> DNAs können auch <RTI ID=25.3>für</RTI> diese Aufgabe verwendet werden. Eine vorhergehende Aufreinigung der zu analysierenden RNAs, die meist eine <RTI ID=25.4>zusätzliche</RTI> Fehlerquelle darstellt, ist hierzu nicht notwendig. < RTI ID=25.5> Ahnlich< /RTI> as is the case for < RTI ID=25.6> erfindungsgemässen< /RTI> < RTI ID=25.7> PathoCheckTM Verfahren< /RTI> werden die zu untersuchenden mRNAs mit einem Überschuss eines modifizierten z. B. biotinylierten Anchor-Oligonukleotids mit genspezifischen einzelsträngigen Antisense-DNAs hybridisiert und an einer z. B. Streptavidin-beschichteten festen Phase immobilisiert. After the washing out of all proteins, not relevant nucleic acids and other impurities goal mRNAs with a set of direct or indirect fluorescence-labeled Splinkersequenzen, which to another part this is more complementary mRNAs, is proven. Durch die Verwendung verschiedener genspezifischer Antisense-DNAs und unterschiedlich markierter Nachweissplinker-Oligonukleotide kann die Expression mehrerer Gene gleichzeitig analysiert werden. The whole method < RTI ID=25.8> left; /RTI> without large expenditure < itself; RTI ID=25.9> vollständig< /RTI> automate. < RTI ID=25.10> Sofern< /RTI> die zu analysierenden Gewebe nicht übermässig viel RNase enthalten, reicht eine Lyse in chaotropen Puffern und/oder Zugabe von RNasin aus, die Integrität der RNAs zu <RTI ID=25.11>gewährleisten.</RTI> Wenn maximale <RTI ID=25.12>Sensitivität</RTI> wichtiger ist als der gleichzeitige Nachweis verschiedener mRNAs in einem Reaktionsansatz, können anstelle der fluoreszenzbasierten Nachweisreagenzien auch <RTI ID=25.13>Antikörper</RTI> eingesetzt werden, welche an ein polyvalentes Sekundärreagenz binden wie ein anti-Maus-Ig-Peroxidase-Polymer. These complexes then in an enzyme reaction downstream detected z. B. by the chemistry luminescence developing with the shifting of a suitable substrate. <RTI ID=25.14>Für</RTI> particularly < RTI ID=25.15> häufig< /RTI> made testings can do appropriate < RTI ID=25.16> GProTM Kits< /RTI> with synthetic KontrollmRNAs as quantitative standards already finished < RTI ID=25.17> konfektioniert< /RTI> become.

10. Allelidentifizierung durch hybridvermittelte Ligation (LIMATM ; Ligation mediated Identification OF Mutant < RTI ID=25.18> Allele) < /RTI> Particularly in the Pränataldiagnostik of hereditary diseases, in addition, to the identification of the individual < RTI ID=25.19> Sensitivität< /RTI> opposite different medicaments the Genotyp of certain alleles must be determined. Usually < RTI ID=25.20> geschieht< /RTI> this by an PCR amplification which can be examined of the Lokus from the genomischen DNA and following < RTI ID=26.1> Restriktionsanalyse< /RTI> or < RTI ID=26.2> Sequenzierung.< /RTI> In the first case this causes a gelelektrophoretische < RTI ID=26.3> Auftrennung< /RTI> the Restrik< RTI ID=26.4> tionsfragmente, < /RTI> those is not so easily automizable. That applies also in the second case, < RTI ID=26.5> sofern< /RTI> not with the Chipsequenzierungsmethode one works, which did not develop however yet. Also for this aspect leave themselves < RTI ID=26.6> erfindungsgemäss< /RTI> manufactured DNA fragments use. A condition for this is that it itself around well-known, molecularly identified alleles to act must. < RTI ID=26.7> erfindungsgemäss< /RTI> manufactured Anchor Oligonukleotid is then designed in such a way that it hybridizes to a gene region, which lies directly before the mutation. A further Oligonukleotid, which < or several; RTI ID=26.8> fluoreszierende< /RTI> Tags < RTI ID=26.9> contains, < /RTI> hybridized to bordering the directly < RTI ID=26.10> 3 ' - Region< /RTI> the gene, so that the two free ends < RTI ID=26.11> erfindungsgemäss< /RTI> manufactured Anchor Oligonukleotid DNA and the fluorescence-labeled Oligonukleotids with more constant < RTI ID=26.12> Hybridbildung< /RTI> to lie directly next to each other < RTI ID=26.13> kommen< /RTI> and to be ligiert with one another < RTI ID=26.14> können.< /RTI> < RTI ID=26.15> Sofern< /RTI> the sequence in this place deviates, does not come it not to the accumulation of the ends and thus also not from the Ligation. Instead z can. B. a Oligonukleotid to the appropriate mutated sequence, labeled with another fluorescence, it binds whereby another marker at the biotinylierten Anchor is ligiert. The bound in each case fluorescence coloring materials and with it the respective alleles are identified by suggestion for laser. To < RTI ID=26.16> Erhöhung< /RTI> < RTI ID=26.17> Sensitivität< /RTI> < RTI ID=26.18> erfindungsgemässen< /RTI> Method can also in this case one < RTI ID=26.19> asymmetrische< /RTI> PCR become upstream, which those which can be examined Lokus amplifiziert. With uniform reaction conditions < RTI ID=26.20> für< /RTI> the PCR and hybridizing are < it; RTI ID=26.21> possible, < /RTI> to determine several different alleles simultaneous from a sample.

11. Direct interaction analysis of protein array < RTI ID=26.22> (LISPATN< /RTI>
With the success of the " human Genome Project " stands as one < RTI ID=26.23> nächsten< /RTI> Those gave up Classification that approx. 50.000 human genes on. Not only in the basic research, but also in the rapidly developing further field of the molecular medicine is important it to understand, what these genes do, like it in which situations with one another cooperate, which proteins, peptides or < RTI ID=26.24> niedermolekularen< /RTI> Materials to which other proteins bind etc.

A first reference point for such co-operation between proteins is usually a direct physical contact of the respective gene products. Around such connections in vitro on that
Protein level examine to be able, < RTI ID=26.25> benötigt< /RTI> one usually up-cleaned protein < RTI ID=26.26> präparationen.< /RTI> With 50.000 proteins this is however not so easily < RTI ID=26.27> möglich.< /RTI> One manages therefore mostly with genetic methods such as z. B. the so-called " Yeast Two hybrid screen ", over < RTI ID=27.1> mögliche< /RTI> To identify interaction partner. This method so far also used is so successful, is nevertheless

extremely < it; RTI ID=27.2> artifact-susceptibly, < /RTI> pedantically and for < RTI ID=27.3> Komplexität< /RTI> now < RTI ID=27.4> anstehenden< /RTI> Task unsuitable. This task can with a combination < RTI ID=27.5> erfindungsgemässen< /RTI> Method, < RTI ID=27.6> SloningTM method, < /RTI> and < RTI ID=27.7> erfindungsgemässen< /RTI> < RTI ID=27.8> EasyProTM Verfahren< /RTI> in connection with a biological chip to be accomplished. Composition of an automation the complete 50,000 genes can synthesized, exprimiert and with a suitable day to < RTI ID=27.9> Immobilisierung< /RTI> in < RTI ID=27.10> Reaktionskammem< /RTI> a biological chip to be provided. < RTI ID=27.11> Für< /RTI> Connection studies with a fluorescence-labeled protein or one < RTI ID=27.12> niedermolekularen< /RTI> chemical connection is a quantity of < RTI ID=27.13> 107< /RTI> to < RTI ID=27.14> 108< /RTI> < RTI ID=27.15> Molekülen< /RTI> usually sufficiently. In < RTI ID=27.16> Kavitäten< /RTI> from 100 x 200 x 60 < RTI ID=27.17> am< /RTI> can < RTI ID=27.18> 1< /RTI> Nano-litre of a protein solution to be deposited, this < RTI ID=27.19> entspricht< /RTI> with 100 kD a protein and a concentration of 5 mg/ml approx. 3 x < RTI ID=27.20> 10 ' < /RTI> < RTI ID=27.21> Molekülen.< /RTI> One assumes < RTI ID=27.22> tatsächliche< /RTI> < RTI ID=27.23> Bindungskapazität< /RTI> per < RTI ID=27.24> Kavität< /RTI> with approx. 1% of this value lie, are also with relatively large proteins still sufficiently material available. If the particulars < RTI ID=27.25> Kavitäten< /RTI> approx. 30 < RTI ID=27.26> um< /RTI> lie apart, then < RTI ID=27.27> könnte< /RTI> the entire library of 50.000 proteins on a chip of only 20 < RTI ID=27.28> cm2< /RTI> are accommodated. A laser measures the fluorescence in all < RTI ID=27.29> Kavitäten< /RTI> before and after the connection of the fluorescence-labeled < RTI ID=27.30> Goal molecule, < /RTI> from which one < RTI ID=27.31> Stärke< /RTI> the interaction berechnt. < RTI ID=27.32> Cavities, < /RTI> in those day is only presented, serves as nonspecific control. With the help of such protein arrays for example so far ascertainable connections of < do not leave themselves; RTI ID=27.33> Arzneimitteln< /RTI> to cellular proteins or signal transduction cascades also complicated detect < RTI ID=27.34> nachvollziehen.< /RTI>

12. Parallel analysis of mRNAs with immobilized nucleic acid arrays < RTI ID=27.35> (PAMINATM) < /RTI> One of the emphasis of the modern medicament research exists in the purposeful interference into the expression of individual genes. In addition the influence of new active substances on the expression of other genes must < RTI ID=27.36> möglichst< /RTI> comprising to be examined. With < RTI ID=27.37> Signal transmission processes, < /RTI> the cell differentiation or when illness induce metabolic changes a whole cascade of different genes is often switched off on-or.

Due to < RTI ID=27.38> Komplexität< /RTI> the Genexpression in < RTI ID=27.39> höheren< /RTI> Organisms is up to now practically < it however; RTI ID=27.40> possible, < /RTI> to analyze more than one handful of genes simultaneous. With that Sequenzierung of the human gene COM will be created in future however the basic conditions for a comprising parallel analysis of the entire Genexpression of a cell. From the available < RTI ID=28.1> Sequenzinformationen< /RTI> can by computerized sequence comparisons first the regions in the individual genes be identified, which < RTI ID=28.2> geringste< /RTI> Homologie among themselves exhibit, thus < RTI ID=28.3> höchsten< /RTI> Degree < on; RTI ID=28.4> Spezifität< /RTI> < RTI ID=28.5> für< /RTI> < RTI ID=28.6> jeweilige< /RTI> Gene. From these gene sections towards-specific, < can; RTI ID=28.7> einzelsträngige< /RTI> Anti-scythe Anchor DNAs to be derived, which becomes immobilized in an array on a biological chip.

The Antisense Anchor DNAs can be conceived in such a way that the fusing temperatures < RTI ID=28.8> sämtlicher< /RTI> RNA/DNA hybrids in a close window lie. Through < RTI ID=28.9> Hybridisierung< /RTI> the entire RNA, and/or. the polya+ RNA of the cells to this array under conditions of maximum Stringenz, which can be examined, in everyone are < RTI ID=28.10> Kavität< /RTI> the biological chip RNA/DNA hybrid produces, if the corresponding < RTI ID=28.11> mRNA< /RTI> one exprimiert. In 2. Step the immobilized Antisense Anchor DNAs at the hybridized RNA Template becomes by treatment with a RNaseH Reversen Transkriptase and modified nucleotides, which are preferably fluorescence-labeled, elongated. After several washing processes, in order to separate inkorporierte nucleotides, the cDNA Reaktionsprodukte analog can to the described < RTI ID=28.12> LISPATM Technik< /RTI> by laser scanning of the particulars < RTI ID=28.13> Kavitäten< /RTI> are measured.

< RTI ID=28.14> Ausführungsbeispiele< /RTI> 1. Preparation Anchor and of the Splinkeroligonukleotide Anchor and the Splinker Oligonukleotide became after by Sinha the N. D., < RTI ID=28.15> Biernat< /RTI> J., McManus J., Köster H., Nucleic Acids Res, 1984 June, 12: 11,4539-57 described standard techniques manufactured or by a synthesis in large ruler, which was successively quartered or by simultaneous synthesis < RTI ID=28.16> on Cellulosemembranen.< /RTI>

2. Tag with a modification modifications in the Oligonukleotiden were < with standard techniques; RTI ID=28.17> durchgeführt.< /RTI>

3. Coupling to the matrix to 10 < RTI ID=28.18> ul< /RTI> Streptavidin coupled magnetized Beads (MERCK) in a total volume of 50 < RTI ID=28.19> pl in lxTE/1< /RTI> M < RTI ID=28.20> NaCl, < /RTI> pH 7,5 20-200 pmol Biotin labeled kinasierte Anchor became Oligonukleotide < RTI ID=29.1> zugesetzt< /RTI> and 30 min at ambient temperature on a scooter < RTI ID=29.2> inkubiert.< /RTI>

Subsequently, bound Anchor Oligonukleotide was not < by a threefold buffer change of; RTI ID=29.3> ever 500 pl lxTE, < /RTI> pH 7,5 away-washed.

3. First Ligationsschritt the Ligation took place with < RTI ID=29.4> 4 C, < /RTI> < RTI ID=29.5> 16 C, < /RTI> Ambient temperature and/or. < RTI ID=29.6> 37 C< /RTI> (Standard < RTI ID=29.7> 16 C) < /RTI> in a volume of 50 < RTI ID=29.8> ul< /RTI> in < RTI ID=29.9> 1< /RTI> x Ligasepuffer (Boehringer Mannheim) with 1 to 5 units T4 DNA ligase (Boehringer Mannheim or new England bio lab) < RTI ID=29.10> für< /RTI> 15 to 60 minutes. < RTI ID=29.11> Für< /RTI> the Ligation was usually used 20 pmol phosphoryliertes Anchor Oligonukleotid. To < RTI ID=29.12> 5 ' - Ende< /RTI> phosphorylierte Splinker Oligonukleotide was admitted in 1,5 to 5fachem molecular surplus. < after the reaction ligase and not ligierte Splinker Oligonukleotide by three buffer changes of ever 500; RTI ID=29.13> 1ll lxTE, < /RTI> pH 7,5 away-washed. Afterwards to the washed Beads 40 one < RTI ID=29.14> ul< /RTI> a Restriktionsmixes given, which < the Splinkerspezifi restriction enzyme; RTI ID=29.15> Eco31I< /RTI> in < RTI ID=29.16> 1,25x< /RTI> Restriction buffer (buffer A von Boehringer one home and/or. Buffer 4 of new England bio labs) contained. Afterwards as managing described one washed.

5. Second Ligationsschritt

Four further Ligationen with further Splinker Oligonukleotiden became after under point
4 < RTI ID=29.17> aufgeführten< /RTI> Regulation < RTI ID=29.18> durchgeführt.< /RTI>

6. Transposition
After the 5. Ligation became after the washing mixes the Anchor specific
Restriction enzyme < RTI ID=29.19> Esp3I< /RTI> and/or. BpiI in the appropriate manufacturer-specific buffers added and 30 to 60 minutes with < RTI ID=29.20> 37 C< /RTI> < RTI ID=29.21> inkubiert.< /RTI> After the reaction the complete became
Mix with the cut off aufligierten Splinker Oligonukleotiden far away, in a separate reaction container 15 minutes with < RTI ID=29.22> 65 C< /RTI> heat-treated to inactivate over the restriction enzyme and then into a further reaction container with according to aufligierten Anchor Oligonukleotide coupled at magnetized Streptavidin Beads ligiert.

7. Restriction control of ligierter fragments
As a check of the correct value of the cut Splinker Oligonukleotide 5 one < RTI ID=29.23> ul< /RTI> Aliquot of the reaction on a 18%igen 1xTBE-Polyacrylamidgel isolated, with 0,01% < RTI ID=30.1> SYBR GoIdTM< /RTI> in < RTI ID=30.2> 1< /RTI> x TBE for 10 min < RTI ID=30.3> angefärbt< /RTI> and with UV light visibly made. On such a gel length differences can be recognized by 1-2 bases.